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PRINCIPAL INVESTIGATOR: Robert A. Vigersky, COL MC

CONTRACTING ORGANIZATION:

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| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT The hypothesis to be tested was that there are allelic variations of some genes that make the development of diabetes-related complications more likely in patients who carry them than those who do not. The 3 major complications to be evaluated were diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy. This was an observational study in which the investigators obtained DNA samples from the blood of patients with one or more of these complications and from as many their first-degree relatives as possible for testing in the laboratory of Dr. Massimo Trucco in the Rangos Research Center at the Children's Hospital of Pittsburgh (CHOP). Dr. Trucco is an internationally known immunologist and respected leader in genetic research in diabetes. He evaluated these samples by studying candidate genes selected <i>a priori</i> and testing for transmission/disequilibrium – a standard for analysis of linkage between a candidate gene and a specific disease. | | | | | |
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Introduction

Although deaths today from the acute effects of diabetes are rare, the associated vascular, retinal, neurological and renal complications are responsible for high levels of morbidity and mortality in diabetes. However, it has been observed that only a subset of people with diabetes appear to be susceptible to the development of diabetes-related complications, i.e., nephropathy, autonomic neuropathy, and retinopathy, and there is data to suggest that there is a genetic component to this increased susceptibility. This investigation tested the hypothesis that there are allelic variations of some genes that make the development of diabetes-related complications more likely in patients who carry them than in those who do not. Initial emphasis was on the examination of candidate gene analysis in families for diabetic nephropathy, autonomic neuropathy, and retinopathy.

Body

This study, "Genetic Screening in Diabetes", was an observational study in which COL Vigersky and his research team obtained DNA samples from the blood of patients with type 1 or 2 diabetes who had at least one of three diabetic complications (as specified in SF298) and from as many of their first-degree relatives as possible for genetic testing. The study was conducted at WRAMC for DEERS-eligible subjects and at the White Flint Professional Building in Kensington, Maryland for non-DEERS-eligible subjects. All subjects completed a medical history, a quality of life questionnaire, a physical examination, blood and urine sampling and analysis, and additional procedures to rule out diabetes and the presence or absence of the three diabetes-related complications that are being studied. All blood samples will be typed and examined to evaluate if there are reasonable candidate genes that contribute to the genetic susceptibility and/or development of diabetic nephropathy, neuropathy, and retinopathy. Sixty-one probands and 62 family members completed the study.

Key Research Accomplishments

- Samples from the 124 consented subjects have been sent to the Rangos Research Center, University of Pittsburgh, Pittsburgh, PA for genetic analysis.
- During the period of this report, the RRC focused their effort on recruitment of additional subjects for the Type 1 Diabetic Nephropathy (T1DN) study. As a result, they have identified a genetic signal on Chromosome 13q with a p-value for T1DN less than $2E-07$. While this is an excellent p-value for association it does not exceed the Bonferroni correction for multiple testing. In the genome-wide association scan (GWAS) that was used to compare the genetics of T1DN cases and T1D controls they originally genotyped roughly 500,000 single nucleotide polymorphisms. This number of independent tests for gene association resulted in a threshold for

genome-wide significance of $1E-07$ (i.e. 0.05 divided by $500,000$). Their value is close but is not yet significant.

- In order for the observed p-value to become significant RRC needs to recruit additional subjects to the study. The ideal cohort would be an independent group of T1DN cases and T1D controls that is roughly the same number ($N=1,000$ cases and $N=1,000$ controls) as was used in the original genome-wide association study (GWAS).
- RRC needs approximately 800 DN samples to confirm the results, but combined efforts from all sites have resulted in less than 200 samples.
- RRC will use samples sent from WRAMC to confirm their findings for T1DN and may use the samples later to identify possible associations between specific genes and diabetic retinopathy and neuropathy.

Reportable Outcomes & Conclusions

- There are no findings or conclusions to date from the samples we have sent to Rangos Research Center,

Summary

- Enrollment for this study was closed on 3 August 2009.
- Since June 2008, the study had been conducted under two no cost extensions. The first was submitted in June 2008 and approved in October 2008, the second was submitted in February 2009 and approved in March 2009.
- Attempts to obtain additional funding for this study were unsuccessful. In July, 2009, it was determined that the RRC needed far more samples than WRAMC was likely to provide. Given the lack of funding and the prior enrollment rate, the PI made the decision to close the study. The last proband was enrolled and completed the study on August 3, 2009.

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Appendices

Appendix A: Candidate genes for Diabetic Complications (see legend)

| EXTRACELLULAR MATRIX | SYMBOL | CHROMOSOME |
|--|--------|---------------|
| collagen 4A1 | COL4A1 | 13q34 |
| collagen 4A2 | COL4A2 | 13q35 |
| collagen 4A3 | COL4A3 | 2q36-q37 |
| collagen 4A4 | COL4A4 | 2q36-37 |
| collagen 4A5 | COL4A5 | Xq22 |
| collagen 4A6 | COL4A6 | Xq22 |
| fibronectin 1 | FN1 | 2q34 |
| integrin, alpha 2 | ITGA2 | 5q23-q31 |
| integrin, alpha V | TGA5 | 12q11-q13 |
| integrin, beta 1 | ITGB1 | 10p11.2 |
| laminin A4 | LAMA4 | 6q21 |
| laminin B1 | LAMB1 | 7q22 |
| laminin B2 | LAMB2 | 3p21.1 |
| nidogen (entactin) | NID | 1q43 |
| ENZYMES | | |
| #aldose reductase | ALDR | 7q35 |
| *angiotensin converting enzyme | ACE | 17q23 |
| cathepsin B | CTSB | 8p22 |
| endothelin converting enzyme 1 | ECE-1 | 1p36.1 |
| metalloproteinase-3 (stromelysin) | MMP3 | 11q23 |
| *methylenetetrahydrofolate reductase | MTHFR | 1p36.2 |
| *paraoxonase 1 | PON1 | 7q21.1 |
| protein kinase C, alpha | PRKCA | 17q22-q23.2 |
| protein kinase C, beta 1 | PRKCB | 16p11.2 |
| renin | REN | 1q32 |
| tissue inhibitor of metalloproteinase 2 | TIMP-2 | 17q25 |
| tissue inhibitor of metalloproteinase 3 | TIMP-3 | 22q12.1-q13.2 |
| CYTOKINES & GROWTH FACTORS | | |
| fibroblast growth factor 2 (basic) | FGF2 | 4q25-q27 |
| insulin-like growth factor 1 | IGF1 | 12q22-q24.1 |
| insulin-like growth factor binding protein-1 | IGFBP1 | 7p14-p12 |
| platelet-derived growth factor beta | PDGFB | 22q12.3-q13.1 |

| | | |
|-------------------------------------|-------|---------------|
| transforming growth factor-beta1 | TGFB1 | 19q13.1-q13.3 |
| transforming growth factor-beta2 | TGFB2 | 1q41 |
| transforming growth factor-beta3 | TGFB3 | 14q24 |
| *vascular endothelial growth factor | VEGF | 6p21.1 |

HORMONES

| | | |
|-------------------------------------|------|----------|
| atrial natriuretic factor (peptide) | NPPA | 1p32.6 |
| adrenomedullin | M | 11 |
| angiotensinogen | AGT | 1q42-q43 |
| preproendothelin | EDN1 | 6p24-p23 |

RECEPTORS

| | | |
|--|----------|-------------|
| AGE receptor | AGER | 6p21.3 |
| angiotensin-2 receptor 1A | AT2R1 | 3q21-q25 |
| *beta-adrenergic receptor | ADRB2 | 5q31.1-qter |
| endothelin receptor A | EDNRA | 12q22.1 |
| endothelin receptor B | EDNRB | 13q22 |
| insulin-like growth factor 1 receptor | IGF1R | 15q25-q26 |
| insulin receptor-related receptor | INSRR | 1q21-q22 |
| PDGF receptor-beta | PDGFRB | 5q31-q32 |
| #Toll-like receptor 4 | TLR4 | |
| transforming growth factor-beta receptor II | TGFBR2 | 3p22 |
| transforming growth factor-beta receptor III | TGFBR3 | 1p33-p32 |
| #tumor necrosis factor receptor 4 | TNFRSF1B | 1p36 |

TRANSCRIPTION FACTORS

| | | |
|-------|-----|---------------|
| c-fos | FOS | 14q24.3 |
| c-jun | JUN | 1p32-p31 |
| c-myc | MYC | 8q24.1-q24.13 |

OTHERS

| | | |
|---|---------------|------------|
| apolipoprotein-E | APOE | 19q13.2 |
| glucose transporter-1; solute carrier family 2 | GLUT1, SCL2A1 | 1p35-p31.3 |
| Na ⁺ /H ⁺ antiporter; solute carrier family 9 | NHE1; SLC9A1 | 1p36.1-p35 |

Legend:

* Signifies candidate gene for retinopathy
Signifies candidate gene for neuropathy
All others are candidate genes for nephropathy

Appendix B: Supporting Data

The information and new technology generated by the Human Genome Project are making it possible to perform large-scale, comprehensive, gene expression analyses. Technical advances in DNA microarray have made it possible to study hundreds to thousands of transcripts simultaneously. The identity and function of many transcripts are already available in public database such as dbEST and Unigene. Together, these advances should allow a different approach to studying the genetic basis of complex diseases. Instead of starting from genetic variation detected at the DNA level, and then determining whether that variation plays a role in gene expression and protein function, we can also study the gene expression pattern, then look for the genetic variation.